CLAIMS

We claim:

1. A method of identifying variant recombinases that mediate recombination at variant recombination sites, the method comprising,

(a) bringing into contact

a mutant recombinase,

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase,

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

Subc1) 3. The method of claim 1 wherein the first and second recombination sites cannot be recombined by non-mutant recombinase to a significant extent.

4. The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

11. The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth

recombination sites inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. A method for producing site-specific recombination of DNA, comprising,

contacting a variant recombinase identified by the method of claim 1 with first and second DNA sequences,

wherein the first DNA sequence comprises a first recombination site and the second DNA sequence comprises a second recombination site,

wherein the variant recombinase mediates recombination between the first and second recombination sites thereby producing the site specific recombination.

25. The method of claim 24 wherein the first recombination site, the second recombination site, or both, are variant recombination sites.

26. The method of claim 24, wherein the first and second DNA sequences are connected by a pre-selected DNA segment.

27. The method of claim 26, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. The method of claim 27 further comprising contacting the variant recombinase with a fourth DNA sequence comprising a third recombination site, wherein the second and fourth DNA sequences are connected by a second pre-selected DNA segment.

30. The method of claim 29 wherein the first recombination site is a variant recombination site recognized by the variant recombinase and not by wild type recombinase, and wherein the second and third recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. The method of claim 30 further comprising, prior to contacting the variant recombinase with the first, second, and third recombination sites, contacting the recombination sites with wild type recombinase, thereby producing site specific recombination between the second and third recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. The method of claim 26 wherein the first and second recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. The method of claim 33, wherein the first and second recombination sites are variant recombination sites recognized by the variant recombinase.

35. The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

36. The method of claim 24, wherein the second and third DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. The method of claim 36, wherein the first and second recombination sites are variant recombination sites recognized by the variant recombinase.

38. The method of claim 24 wherein the second DNA sequence includes a label, wherein recombination between the first and second recombination sites associates the label with the first DNA sequence.

39. The method of claim 38 wherein the first DNA sequence is a large circular DNA molecule.

40. The method of claim 24 wherein recombination occurs in a cell.

41. The method of claim 40 wherein the variant recombinase is contacted with the first and second DNA sequences by introducing into the cell a third DNA sequence comprising DNA encoding the variant recombinase.

42. The method of claim 41, wherein the third DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

44. The method of claim 43 wherein the multicellular organism is a plant, an animal, or a mammal.

45. The method of claim 40, wherein the first and second DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

46. The method of claim 45 wherein the cell is in a multicellular organism.

47. The method of claim 45, wherein the pre-selected segment is an undesired marker or trait gene.

48. The method of claim 24 wherein the variant recombinase is contacted with the recombination sites *in vitro*.

49. The method of claim 48 wherein the method further comprises introducing the recombined DNA into a cell.

50. A method for cloning large DNA fragments, the method comprising

concatenating DNA fragments to be cloned with vector arms, wherein each vector arm comprises a recombination site, wherein the DNA fragments and vector arms alternate in the concatemer,

introducing the concatemer into a cell expressing a variant recombinase identified by the method of claim 1, wherein the recombinase mediates recombination of the recombination sites thereby generating circles each containing a DNA fragment and a vector arm.

51. A method for cloning large DNA fragments, the method comprising

ligating a DNA fragment to be cloned to vector arms, wherein each vector arm comprises (i) a blunt end, (ii) another end which is compatible with an end of the DNA fragment to be cloned, and (iii) a recombination site, wherein concatemers are not formed, and

introducing the ligated DNA fragment and vector arms into a cell expressing a variant recombinase identified by the method of claim 1.

52. A method for cloning large DNA fragments, the method comprising

ligating a plurality of DNA fragments to be cloned with a plurality of first and second vector arms, wherein each first vector arm comprises two ligatable ends, wherein each second vector arm comprises a recombination site and one non-ligatable end,

wherein, following ligation, the DNA fragments and first vector arms alternate in concatemers, wherein the concatemers are flanked by second vector arms,

introducing the concatemers into a cell expressing a variant recombinase identified by the method of claim 1, wherein the recombinase mediates recombination of the recombination sites thereby generating circles containing the DNA fragments.

53. A variant recombinase identified by the method of claim 1.

54. A nucleic acid molecule encoding a variant recombinase identified by the method of claim 1.

55. The nucleic acid molecule of claim 54 wherein the nucleic acid molecule is a plasmid.

56. A cell containing the nucleic acid molecule of claim 54.

57. The cell of claim 56 wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

58. The cell of claim 57 wherein the multicellular organism is a plant, an animal, or a mammal.

59. A nucleic acid molecule having at least one variant recombination site, wherein the variant recombination site is recognized by a variant recombinase identified by the method of claim 1 and is not recognized by wild type recombinase.

60. The nucleic acid molecule of claim 59 wherein the recombination site is not recognized by wild type recombinase.

61. The nucleic acid molecule of claim 59, wherein a first recombination site and a second recombination site are connected by a pre-selected DNA segment.

62. The nucleic acid molecule of claim 59 wherein the nucleic acid molecule is a plasmid.

63. A cell containing the nucleic acid molecule of claim 59.